

AD \_\_\_\_\_

Award Number: DAMD17-99-1-9170

TITLE: Roles of BRCA2 Gene in Homologous Recombination and  
Genomic Stability

PRINCIPAL INVESTIGATOR: David J. Chen, Ph.D.

CONTRACTING ORGANIZATION: University of California  
Berkeley, California 94720

REPORT DATE: September 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20020717 041

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 074-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE September 2001	3. REPORT TYPE AND DATES COVERED Annual (1 Sep 00 - 31 Aug 01)		
4. TITLE AND SUBTITLE Roles of BRCA2 Gene in Homologous Recombination and Genomic Stability		5. FUNDING NUMBERS DAMD17-99-1-9170		
6. AUTHOR(S) David J. Chen, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of California Berkeley, California 94720  E-Mail: DJChen@lbl.gov		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSORING / MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES Report contains color				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words)  The <i>Brca2</i> tumor suppressor gene is important for maintaining genomic integrity because, at least in part, of a role in homologous recombinational repair. <i>Brca2</i> is presumed to function in homologous recombination through its interactions with Rad51. Both exons 11 and 27 of <i>Brca2</i> code for domains that interact with Rad51; exon 11 codes for eight BRC motifs, while exon 27 codes for a single, distinct interaction domain. Deletion of all the Rad51-interacting domains causes early embryonic lethality in mice. A less severe phenotype is seen in mice with deletions that preserve some, but not all, of the BRC motifs. These mice can survive past weaning, but are runted, infertile, and die very young from cancer. Cells derived from such mice are hypersensitive to some genotoxic agents and exhibit chromosomal instability. Here we present analysis of mice and cells with a deletion of the single Rad51-interacting region encoded by exon 27 (all the BRC motifs are preserved). This mutation is called <i>brca2<sup>lexl</sup></i> . Mice homozygous for <i>brca2<sup>lexl</sup></i> exhibit a shorter life span compared to control littermates, possibly due to an early onset of cancer and sepsis. No other phenotype was observed in these animals; therefore, the <i>brca2<sup>lexl</sup></i> mutation is less severe than those with larger COOH-terminal truncations that delete some of the BRC motifs. However, at the cellular level, the <i>brca2<sup>lexl</sup></i> mutation causes reduced viability, extreme sensitivity to the DNA interstrand cross-linking agent mitomycin C, and gross chromosomal instability, much like the more severe truncations..				
14. SUBJECT TERMS Breast Cancer			15. NUMBER OF PAGES 11	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT  Unlimited	

## Table of Contents

Cover.....	1
SF 298 .....	2
Table of Contents .....	3
Introduction.....	4
Body .....	4-7
Conclusions .....	7
Key Research Accomplishments.....	7
Reportable Outcomes .....	8
References .....	9
Appendices .....	10-11

## Introduction

The BRCA2 protein has been shown to physically interact with the DNA repair and homologous recombination protein RAD51, both in vitro and in vivo (Chen *et al.*, 1998b; Katagiri *et al.*, 1998; Marmorstein *et al.*, 1998; Mizuta *et al.*, 1997; Sharan *et al.*, 1997; Wong *et al.*, 1997) suggesting that BRCA2 participates in a recombination complex during cell division and DNA repair processes. Shortly after exposure of normal cells to ionizing radiation, RAD51 protein relocates to form discrete nuclear foci, which have been proposed to represent the assembly of multiprotein recombinational repair complexes at sites of DNA damage (Chen *et al.*, 1998a; Chen *et al.*, 1999; Haaf *et al.*, 1995). In Capan-1 human cells, which lack an intact *BRCA2* gene, formation of RAD51 foci is severely impaired (Yuan *et al.*, 1999). The latter cells are also hypersensitive to ionizing radiation and to drugs that cause DNA double-strand breaks, and show reduced repair of DNA double-strand breaks after radiation exposure (Abbott *et al.*, 1998). If loss of the interactions between BRCA2 and RAD51 results in reduced capacity for homologous repair of DNA double-strand breaks or homology-dependent repair of other lesions during DNA replication, this may in turn contribute to genomic instability, and result in the eventual mutation of genes required for control of cell growth and division.

Morimatsu has reported the generation of embryonal stem (ES) cells in which both alleles of the *BRCA2* gene have been truncated by gene targeting, using two different targeting vectors (Morimatsu *et al.*, 1998). Both truncations delete exon 27, which encodes the extreme carboxy-terminal RAD51-interaction domain of the BRCA2 protein. The two targeted alleles have been designated *BRCA2<sup>lex1</sup>* and *BRCA2<sup>lex2</sup>*. *BRCA2<sup>lex1/lex2</sup>* ES cells are hypersensitive to ionizing radiation but not to ultraviolet radiation. The *BRCA2<sup>lex1/lex2</sup>* ES cells were used to generate primary mouse embryonal fibroblasts (MEF). Primary *BRCA2<sup>lex1/lex2</sup>* MEF cells show an impaired growth rate and reduced cloning efficiency as compared to primary *BRCA2<sup>+/+</sup>* MEF, and undergo premature replicative senescence as determined by colony size distribution and serial passage (3T3-equivalent) analysis.

We have reported the characterization of these cells for sensitivity to mitomycin C, which produces DNA interstrand crosslinks; a type of damage thought to require repair through homologous recombination. Here we further characterize the phenotype of mice and mouse embryonic fibroblasts (MEF) with a deletion of exon 27 (designated *brca2<sup>lex1</sup>*). Compared to control mice, the *brca2<sup>lex1</sup>* mice exhibit a decreased life span that may be partially due to an early onset of cancer and sepsis. The *brca2<sup>lex1</sup>* allele exhibits a normal Mendelian pattern of inheritance, indicating no loss of viability in homozygous embryos. In addition, *brca2<sup>lex1</sup>* mice are of normal size and fertile; unlike mice with the larger COOH-terminal truncations that retain some of the BRC motifs. Thus, the *brca2<sup>lex1</sup>* mutation is very mild, and suggests only a modest impairment of Brca2 activity. However, even though *brca2<sup>lex1</sup>* mice exhibit a mild phenotype, MEF bearing this mutation exhibit impaired growth, gross chromosomal instability and severe hypersensitivity to the interstrand crosslinking agent mitomycin C.

## Body

**1) Life Span of *brca2<sup>lex1/lex1</sup>* and Control Mice.** We compared the life span of 52 *brca2<sup>lex1/lex1</sup>* mice to 35 control mice (Figure 1b). Included in the control cohort are *Brca2<sup>+/+</sup>*, *Brca2<sup>+/lex1</sup>*, and *Brca2<sup>+/lex2</sup>* mice. Heterozygote mice were included because there is little phenotypic abnormality in these mice. Mortality came sooner for the *brca2<sup>lex1/lex1</sup>* mice compared to control mice. The onset of mortality is about the same for *brca2<sup>lex1/lex1</sup>* and control mice; 50 - 60 weeks. However, the life span curves began to diverge at about 70 weeks and were significantly different by 93 weeks ( $p < 0.1$ ). This divergence progressed until the

study was terminated, when the youngest mice were 108 weeks, ( $p < 0.01$ ). About 50% of the  $brca2^{lex1/lex1}$  mice die by 89 - 90 weeks while 50% of the control mice die by 104 - 108 weeks.

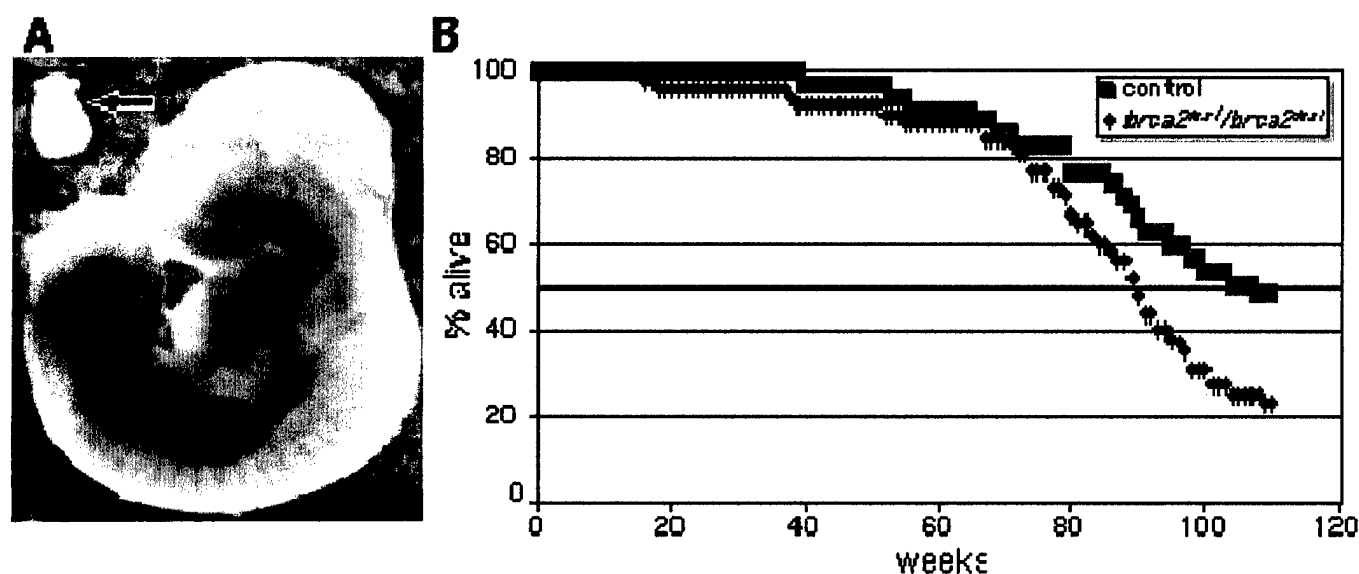


Figure 1. The  $brca2^{lex1}$  and the  $brca2^{lex2}$  mutations in mice. **A.** Day E10.5 embryos harvested from a  $brca2^{lex1/lex2}$  female mated with a  $brca2^{lex1/lex2}$  male. The larger embryo is  $brca2^{lex1/lex2}$  compound heterozygote and appears morphologically normal. The much smaller (partially resorbed) embryo indicated by the arrow at left is a  $brca2^{lex2/lex2}$  homozygote. **B.** Survival curve of  $brca2^{lex1/lex1}$  (blue diamonds) and control (red squares) mice. The percent alive are shown.

**2) Pathology of  $brca2^{lex1/lex1}$  and Control Mice.** Moribund and recently dead mice were observed by necropsy. Additionally, all remaining mice were sacrificed at the point when the survival curve reached 49% for the control population. Abnormal tissues and potential tumors were examined by histopathology, as summarized in Table 1. Both  $brca2^{lex1/lex1}$  and control mice exhibit a wide range of cancers. However, onsets of cancer and sepsis (the latter indicated by reactive immune responses) were somewhat earlier in the  $brca2^{lex1/lex1}$  mice. From weeks 67 to 104, 11  $brca2^{lex1/lex1}$  mice (~20% of the population) developed life-threatening pathology (either cancer or sepsis) while at week 104 the first control mouse exhibited life-threatening disease. The average age of onset for frank neoplasias, considering only tumors that arose through 108 weeks, was 81 weeks for the  $brca2^{lex1/lex1}$  mice as compared to 106 weeks for controls ( $p < .005$ ). The cumulative incidence of cancers and reactive immune responses was significantly higher in the  $brca2^{lex1/lex1}$  cohort compared to the control cohort by week 87 and continued to diverge through 108 weeks ( $p$  values from  $<.05$  to  $.01$ ). Since many of the  $brca2^{lex1/lex1}$  mice were generated after the control mice, not as many  $brca2^{lex1/lex1}$  mice as controls were observed at ages older than 110 weeks (9 compared to 16) or older than 120 weeks (1 compared to 7). For this reason, the frequency of late onset disease is not known for the  $brca2^{lex1/lex1}$  mice and cannot be compared to control mice. Moreover, the differences seen through 108 weeks, while statistically significant, should be interpreted with caution given the small numbers of animals involved. Nonetheless, the shortened life span observed in the  $brca2^{lex1/lex1}$  cohort appears to be due, at least in part, to early onsets of cancer and sepsis.

mouse	genotype	week	histopathology
284H	lex1 / lex1	67	mesenteric abscess
246H	lex1 / lex1	77	colonic adenocarcinoma
107H	lex1 / lex1	79	hepatocellular carcinoma
109H	lex1 / lex1	82	fatty liver with focal inflammation
103H	lex1 / lex1	86	granulomatous inflammation in spleen
264H	lex1 / lex1	87	malignant lymphoma
164H	lex1 / lex1	90	reactive lymphoid hyperplasia in spleen
094H	lex1 / lex1	96	granulomatous inflammation in lymph nodes and skin
281H	lex1 / lex1	98	massive lymphoid hyperplasia in lymph nodes, possibly lymphoma
267H	lex1 / lex1	101	reactive lymphoid hyperplasia in lymph nodes, spleen, liver, kidney
050H	lex1 / lex1	104	hepatocellular carcinoma
256H	lex1 / lex1	114	osteosarcoma
022N	+ / lex2	104	hepatocellular carcinoma
028H	+ / +	108	leiomyosarcoma
051H	+ / lex1	116	hemangiosarcoma
053H	+ / lex1	116	hepatocellular carcinoma
058H	+ / lex1	119	malignant lymphoma
049H	+ / lex1	129	hepatocellular carcinoma

Table 1. Pathology of *brca2*<sup>lex1/lex1</sup> and Control Mice.

**3) To assess the relative effects of DNA damage on chromosomal integrity in wild type and *brca2*<sup>lex1/lex2</sup> immortalized MEF.** Metaphase spreads were prepared 24 hours after exposure to 6 Gy of  $\gamma$ -radiation or to mitomycin C at  $5 \times 10^{-8}$  M. After  $\gamma$ -irradiation, there were significantly increased numbers of chromatid- and chromosome-type abnormalities in *brca2*<sup>lex1/lex2</sup> MEF relative to non-irradiated controls, but this was also true for wild type MEF (See Appendix – Table2). The numbers of chromatid and chromosome aberrations present in *brca2*<sup>lex1/lex2</sup> MEF after irradiation were only slightly higher than in wild type MEF. This is consistent with the modest difference between *brca2*<sup>lex1/lex2</sup> and wild type cells in viability after  $\gamma$ -irradiation, and indicates that *brca2*<sup>lex1/lex2</sup> cells have nearly the proficiency of wild-type cells in repairing the DNA damage induced by ionizing radiation. Mitomycin C at  $5 \times 10^{-8}$  M produced no significant increase in chromatid- or chromosome-type abnormalities in wild type MEF (Table 2), suggesting that cells expressing normal Brca2 were able to repair most of the interstrand crosslinks created. In *brca2*<sup>lex1/lex2</sup> MEF, however, the frequency of chromatid-type aberrations increased more than two-fold after mitomycin C exposure (Table 2.  $p < 0.001$ ).

We wished to determine whether the chromosomal instability seen in immortalized *brca2*<sup>lex1/lex2</sup> MEF arose during the process of immortalization, or already existed in primary cells prior to senescent crisis. We therefore examined early-passage primary MEF for chromosomal abnormality. Two populations of *brca2*<sup>lex1/lex2</sup> MEF and three populations of wild type MEF (Morimatsu *et al.*, 1998) that had been frozen at the first passage after dissociation from embryos (passage 1) were thawed and passaged, with metaphase spreads prepared at each passage. Passage 1 wild-type MEF grew vigorously after thaw and showed no appreciable slowing of growth over at least ten passages afterward (data not shown). However, the *brca2*<sup>lex1/lex2</sup> MEF senesced prematurely in culture, as reported previously (Morimatsu *et al.*, 1998). and could not be expanded beyond the fourth passage. For one of the two primary *brca2*<sup>lex1/lex2</sup> MEF populations examined (281.1), sufficient numbers of metaphase cells for scoring were recovered only at the second and third passages. Scoring of early passage primary MEF for chromosomal aberrations is

summarized in Appendix – Table3. Compared to wild type primary MEF, the *brca2*<sup>lex1/lex2</sup> primary MEF had sharply elevated numbers of chromatid and chromosome aberrations, including gaps, breaks, deletions and exchanges. The *brca2*<sup>lex1/lex2</sup> MEF had higher numbers of chromosomal aberrations even at passage 2, and accumulated further aberrations more rapidly than wild type MEF. By the third passage, approximately half the metaphase cells in *brca2*<sup>lex1/lex2</sup> populations had one or more visible chromosome abnormalities. These results confirm that the chromosomal instability seen in immortalized *brca2*<sup>lex1/lex2</sup> MEF results directly from the *brca2*<sup>lex1/lex2</sup> mutation, and not from additional mutations acquired during or after immortalization.

## Conclusion and Summary

The results obtained here confirm that the extreme COOH-terminal region encoded by exon 27, though not essential to viability, is important for the function of Brca2 at the cellular level. It remains a possibility that the phenotypic effects associated with deletion of exon 27 result from something other than altered interactions with Rad51. In humans, the Brca2 protein is dependent upon nuclear localization signals encoded within exon 27 for transport into the nucleus (Spain *et al.*, 1999). However, this is evidently not the case for mouse Brca2, since the protein encoded by the *brca2*<sup>lex1</sup> allele reaches the nucleus (Moynahan *et al.*, 2001). Thus, in mouse, interaction with Rad51 is the only function so far demonstrated for the region encoded by exon 27. This raises the interesting question of why the Brca2 protein should require multiple Rad51 interaction domains (the BRC repeats as well as the region encoded by exon 27) to be fully functional.

Although the cellular phenotypes of the *brca2*<sup>lex1</sup> truncation and the longer COOH-terminal Brca2 deletions are similar, the mouse phenotypes are very different. Mice homozygous for either of the long COOH-terminal deletions exhibit partial pre-adulthood lethality, infertility, runted growth and high incidence of thymic lymphoma before six months of age (Connor *et al.*, 1997; Patel *et al.*, 1998; Friedman *et al.*, 1998). However, mice that are homozygous for the *brca2*<sup>lex1</sup> truncation appear relatively normal. They grow, reach adulthood, and are fertile much like their control littermates. Nearly all the mice survive beyond one year of age, yet their life span is significantly shorter than control mice, possibly due to early onsets of cancer and sepsis. That the larger COOH-terminal deletions have severely deleterious phenotypes is perhaps unsurprising, given that they eliminate more than a third (Connor *et al.*, 1997), or more than half (Patel *et al.*, 1998) of the Brca2 protein. The large COOH-terminal regions lost in these mutations are likely to contain additional domains with important functions, and so the resulting phenotypes cannot be ascribed with certainty to the loss of Rad51-interacting regions alone. The *brca2*<sup>lex1</sup> allele deletes less than 6% of the protein and leaves all eight of the BRC repeats, as well as the intervening region coded by exons 12 – 26 intact. Moreover, the truncated Brca2 protein encoded by the *brca2*<sup>lex1</sup> allele retains the capacity to bind Rad51, presumably via the BRC repeats (Moynahan *et al.*, 2001). Nonetheless, the *brca2*<sup>lex1</sup> allele produces a qualitatively similar cellular phenotype as the larger deletions. Thus, while the more severe phenotypes seen in mice with longer COOH-terminal deletions may be the result of more severely impaired HRR, it is also possible that they reflect loss of functions unrelated to Rad51. Due to this possibility, the *brca2*<sup>lex1/lex1</sup> mice are an ideal genetic background to evaluate mutations in other genes suspected to functionally interact with Brca2. They may also have utility in testing putative clastogens or carcinogens, since they are predisposed to genetic instability, but exhibit such a mild phenotype.

## Key Research Accomplishments

- The BRCA2<sup>lex2</sup> allele is embryonic lethal when homozygous

- Mice homozygous for either of the long COOH-terminal deletions exhibit partial pre-adulthood lethality, infertility, runted growth and high incidence of thymic lymphoma before six months of age
- BRCA2<sup>lex1/lex2</sup> MEF exhibit chromosomal instability

### **Reportable Outcomes**

- We have generated mouse immortalized BRCA2<sup>lex1/lex2</sup> MEF
- A postdoctoral fellow, Dr. Mark Breenman was hired for this study
- A Scientist, Dr. Yi-Ching Lio is hired to continue this research



## References

- Abbott, D.W., Freeman, M.L. & Holt, J.T. (1998). *J Natl Cancer Inst*, 90, 978-85.
- Chen, J.J., Silver, D., Cantor, S., Livingston, D.M. & Scully, R. (1999). *Cancer Res*, 59, 1752s-1756s.
- Chen, P.L., Chen, C.F., Chen, Y., Xiao, J., Sharp, Z.D. & Lee, W.H. (1998b). *Proc Natl Acad Sci U S A*, 95, 5287-92.
- Connor, F., Bertwistle, D., Mee, P.J., Ross, G.M., Swift, S., Grigorieva, E., Tybulewicz, V.L. & Ashworth, A. (1997). *Nat Genet*, 17, 423-30.
- Cui, X., Brenneman, M., Meyne, J., Oshimura, M., Goodwin, E.H. & Chen, D.J. (1999). *Mutat Res*, 434, 75-88.
- Friedman, L.S., Thistlethwaite, F.C., Patel, K.J., Yu, V.P., Lee, H., Venkitaraman, A.R., Abel, K.J., Carlton, M.B., Hunter, S.M., Colledge, W.H., Evans, M.J. & Ponder, B.A. (1998). *Cancer Res*, 58, 1338-43.
- Haaf, T., Golub, E.I., Reddy, G., Radding, C.M. & Ward, D.C. (1995). *Proc Natl Acad Sci U S A*, 92, 2298-302.
- Katagiri, T., Saito, H., Shinohara, A., Ogawa, H., Kamada, N., Nakamura, Y. & Miki, Y. (1998). *Genes Chromosomes Cancer*, 21, 217-22.
- Marmorstein, L.Y., Ouchi, T. & Aaronson, S.A. (1998). *Proc Natl Acad Sci U S A*, 95, 13869-74.
- Mizuta, R., LaSalle, J.M., Cheng, H.L., Shinohara, A., Ogawa, H., Copeland, N., Jenkins, N.A., Lalande, M. & Alt, F.W. (1997). *Proc Natl Acad Sci U S A*, 94, 6927-32.
- Morimatsu, M., Donoho, G. & Hasty, P. (1998). *Cancer Research*, 58, 000-000.
- Moynahan, M.E., Chiu, J.W., Koller, B.H. & Jasin, M. (1999). *Mol Cell*, 4, 511-8.
- Moynahan, M.E., Pierce, A.J., & Jasin, M. (2001) *Mol Cell*, 7, 263-272
- Patel KJ, Y.V., Lee HS, Corcoran A, Thistlethwaite FC, Evans MJ, Colledge WH, Friedman LS, Ponder BAJ, Venkitaraman AR. (1998). *Molecular Cell*, 1(3), 347-357.
- Sharan, S.K., Morimatsu, M., Albrecht, U., Lim, D.S., Regel, E., Dinh, C., Sands, A., Eichele, G., Hasty, P. & Bradley, A. (1997). *Nature*, 386, 804-10.
- Spain, B.H., Larson, C.J., Shihabuddin, L.S., Gage, F.H., and Verma, I.M., (1999) *Proc Natl Acad Sci U.S.A.* 96, 13920-5
- Wong, A.K.C., Pero, R., Ormonde, P.A., Tavtigian, S.V. & Bartel, P.L. (1997). *J Biol Chem*, 272, 31941-4.
- Yuan, S.S., Lee, S.Y., Chen, G., Song, M., Tomlinson, G.E. & Lee, E.Y. (1999). *Cancer Res*, 59, 3547-51.

## Appendix:

**Table 2.** Spontaneous and damage-induced chromosomal aberrations in immortalized wild type and *brca2*<sup>lex1/lex2</sup> MEF cells.

**Table 3.** Spontaneous chromosomal aberrations in early-passage wild type and *Brca2*<sup>lex1/lex2</sup> primary MEF cells.

Table 2. Spontaneous and damage-induced chromosomal aberrations in immortalized wild type and brca2lex1/lex2 MEF cells.

MEF cells	Metaphases analyzed	Percent aberrant metaphases <sup>3</sup>	Mean aberrations per metaphase <sup>3</sup>	Chromatid aberrations <sup>1</sup>			Chromosome aberrations <sup>2</sup>		
				gaps	breaks	other	int.del.	ter.del.	other total
<b>Wild type</b>									
No treatment	30	50	0.800	5	9	2	11	9	3 1 13
γ-irradiation	30	100	4.33	10	25	29	54	65	5 5 77
Mitomycin C	30	60	0.844	0	8	6	14	8	5 0 13
<b>brca2lex1/lex2</b>									
No treatment	30	97	3.27	7	24	17	41	47	10 0 57
γ-irradiation	30	100	5.07	17	35	24	59	75	0 18 93
Mitomycin C	30	93	4.60	13	66	30	96	27	14 1 42

1. "gaps" defined as discontinuities smaller than the width of the chromatid; "other" comprising isochromatid deletions and exchanges.
2. interstitial deletions; terminal deletions (including breaks); and other (including dicentric and ring chromosomes).
3. calculated without chromatid gaps.

Table 3. Spontaneous chromosomal aberrations in early-passage wild type and Brca2lex1/lex2 primary MEF cells.

MEF cells	Passage	Metaphases analyzed	Percent aberrant metaphases <sup>3</sup>	Mean aberrations per Metaphase <sup>3</sup>	Chromatid aberrations <sup>1</sup>			Chromosome aberrations <sup>2</sup>				
					gaps	breaks	other	total <sup>3</sup>	int.del.	ter.del.	other	total
Wild type												
129.5	P2	50	6	0.06	7	1	0	1	0	2	0	2
	P3	50	6	0.06	13	1	0	1	0	1	1	2
	P4	50	10	0.10	10	1	0	1	0	2	2	4
129.7	P2	50	4	0.06	4	3	0	3	0	0	0	0
	P3	50	8	0.12	1	1	1	2	2	2	0	4
	P4	50	14	0.24	3	5	0	5	2	5	0	7
129.9	P2	50	4	0.04	0	0	0	0	0	2	0	2
	P3	50	8	0.08	1	2	0	2	0	2	0	2
	P4	50	26	0.28	7	3	2	5	3	5	1	9
Brca2 <sup>lex1/lex2</sup>												
283.2	P2	50	24	0.40	22	11	2	13	0	7	0	7
	P3	50	58	2.22	22	50	6	56	23	32	0	55
	P4	42	48	1.02	14	18	4	22	2	27	0	29
281.1	P2	50	52	0.76	5	23	0	23	5	10	0	15
	P3	40	62	1.60	23	35	5	40	5	19	0	24

1. "gaps" defined as discontinuities smaller than the width of the chromatid; "other" comprising isochromatid deletions and exchanges.
2. interstitial deletions; terminal deletions (including breaks); and other (including dicentric and ring chromosomes).
3. calculated without chromatid gaps.